The Self-Identity Protein IdsD Is Communicated between Cells in Swarming Proteus mirabilis Colonies

The self-identity protein, IdsD, is communicated between cells in swarming *Proteus mirabilis* colonies

Authors:

Christina C. Saak and Karine A. Gibbs*

Department of Molecular and Cellular Biology. Harvard University, 16 Divinity Avenue, Cambridge, MA, 02138, USA

* Corresponding author:

Karine A. Gibbs, Ph.D.

Department of Molecular and Cellular Biology

Harvard University

16 Divinity Avenue

Cambridge, MA 02138

Ph: 617-496-1637

Em: *kagibbs@mcb.harvard.edu*

Keywords: self-recognition, kin selection, swarm/swarming, *Proteus mirabilis*, bacterial sociality, motility, type six secretion system, cell-cell communication

Running title: Cell-cell communication of a self-identity protein

Abstract

Proteus mirabilis is a social bacterium that is capable of self (kin) versus non-self recognition. Swarming colonies of this bacterium expand outward on surfaces at centimeter-scale distances due to the collective motility of individual cells. Colonies of genetically distinct populations remain separate while those of identical populations merge. Ids proteins are essential for this recognition behavior. Two of these proteins, IdsD and IdsE, encode identity information for each strain. These two proteins bind *in vitro* in an allele-restrictive manner, and IdsD-IdsE binding is correlated with populations merging whereas a lack of binding correlates with populations separating. Key questions about *in vivo* IdsD and IdsE interactions remained, specifically whether IdsD and IdsE bind within a single cell or whether IdsD-IdsE interactions occur across neighboring cells, and if so, which of the two proteins are exchanged. Here we demonstrate that IdsD must originate from another cell to communicate identity and that this non-resident IdsD interacts with IdsE resident in a recipient cell. Further, we show that unbound IdsD in the recipient cell does not cause cell death and instead appears to contribute to a restriction in the expansion radius of the swarming colony. We conclude that *P. mirabilis* communicates IdsD between neighboring cells for non-lethal kin recognition, suggesting that the Ids proteins constitute a type of cell-cell communication.

Importance statement

We demonstrate that self (kin) versus non-self recognition in *P. mirabilis* entails the cell-cell communication of an identity-encoding protein that is exported from one cell and received by another. We further show that this intercellular exchange impacts swarm colony expansion in a non-lethal manner, thereby adding social communication to the list of potential swarm-related

44 regulatory factors.

45

46

Introduction

Bacteria, such as the swarming bacterium *Proteus mirabilis*, can come together in groups that move rapidly across surfaces. During this swarm migration, *P. mirabilis* exhibits self (kin) versus non-self recognition. Populations of genetically identical organisms merge while populations of genetically different organisms separate and form a visible boundary (1-4). The *ids* operon, encoding the six proteins IdsA to IdsF, is one of the genetic loci responsible for boundary formation (2, 5, 6). Cells lacking the Ids proteins form a boundary with their wild-type parent strain (2). A functional Type VI Secretion System (T6SS) is essential for boundary formation (5, 7), and three Ids proteins (IdsA, IdsB, and IdsD) are exported in a T6SS-dependent manner (5). T6SSs, which are widely distributed among gram-negative bacteria, are machines that can translocate proteins, primarily lethal, from the inside of one cell directly into another (8-28). The action of these transferred effector proteins is inhibited through the binding of an inhibitory immunity protein in the recipient cell (15, 16, 18, 21, 22, 28-30).

In addition to a functional T6SS, the Ids system relies on the interactions between two proteins, IdsD (D) and IdsE (E), which together encode strain-specific identity information (2, 31). D and E each contain a variable region, which is a stretch of amino acids that is generally unique between strains (2, 31). D and E bind *in vitro* when the variable regions of both proteins originate from the same strain. Binding pairs of D and E are termed cognate (31). By contrast, when the variable regions of D and E do not originate from the same strain, these proteins do not bind *in vitro*, and the D-E pair is thus termed non-cognate (31). Interestingly, swarming populations of strains producing cognate D-E pairs merge and thus recognize each other as self; however, swarms of strains producing non-cognate D-E pairs form a visible boundary and are considered non-self (31). How the *in vitro* binding of D and E accounts for *in vivo* boundary

behaviors remains unknown. Both D and E contain transmembrane domains (31). D has been found outside of cells, and its export has been shown to be dependent on a functional T6SS (5). Consistent with this data, D contains the recently described MIX motif, which has been found among multiple T6SS effector proteins; this MIX motif is predicted to identify previously unknown substrates of the T6SS (32). In contrast, E has not been found outside of cells and is predicted to be an integral inner membrane protein (5, 31). Given these data, the prevailing hypothesis is that the Ids proteins comprise a lethal effector/immunity (toxin/anti-toxin) system. Within this model, D is proposed to be delivered to a neighboring cell where it can interact with E; lack of D-E binding might result in cell lethality as seen for other effector/immunity pairs. However, there is no experimental evidence for Ids transfer between cells or for Ids-associated lethality. Further, whether boundary formation results from interactions among the Ids proteins within one cell, or between cells, has not been addressed.

Here we demonstrate that D-E interactions, or lack thereof, do not cause lethality in *P. mirabilis*. Further, we provide evidence that D is communicated from one cell to another in a T6SS-dependent manner and that interactions with E in the recipient cell determine behavior. We also present first evidence that these D-E interactions impact the expansion of a swarming colony. These data demonstrate that kin recognition in *P. mirabilis* entails the cell-cell communication of an identity-encoding protein.

Methods

Bacterial strains and media

Strains and plasmids used in this study are described in Table 1. *P. mirabilis* strains were maintained on LSW$^-$ agar (33). CM55 blood agar base agar (Oxoid, Basingstoke, England) was

used for swarm-permissive nutrient plates. Overnight cultures of all strains were grown in LB broth under aerobic conditions at 37°C. Kanamycin was used at a concentration of 35 μg/ml for plasmid maintenance and was added to all swarm and growth media.

We employed a previously published *ids* expression system (2) in which the entire *ids* locus from *P. mirabilis* strain BB2000 is expressed from a low-copy number plasmid under control of its native promoter (pIdsBB) in a BB2000-derived strain lacking the chromosomal copy of the *ids* operon (Δ*ids*) (2). We engineered alterations to the *ids* locus on the vector; hence, all strains are isogenic except for the encoded *ids* genes.

Plasmid and strain construction

Construction of pIdsBB-ΔE

The pIdsBB-ΔE plasmid was constructed using a 390-basepair (bp) gBlock (Integrated DNA Technologies, Inc., Coralville, IA) containing the last 266 bp of *idsD*, the last 18 bp of *idsE* and the first 106 bp of *idsF* (gBlock sequence:
GCGAACAATTAAAAATGGCAAGTGAAAAAGGTGATTGGAACCCTGAAACAGGTATA
TTTAAATTTAGTTTGGAAGTACAGTCTCAATTAGTAAATACATATTCTGCTTTTGGTG
CACATCCTAATAGCCGTATAGGTATTGAAGATTTATATTGGTATTATCAAGTCAATC
CCGAGGTAACAACACCGATGCGTTATATCAATTGGGGGGGAGATACCCAAGAAAAC
AATCAGCTTTTAGGCTTTATTAACAGTGAGAATATCTAAATCAGGAGAAAGAACACC
ATGCGTAGTTTGGTAAACGGCAGAAAGATTATTTTAGAAAATGATACAACAAATAC
CGGCGGTACCGTACTTACCGGCTCTTCTATTGCTAAACAAACACAAGGGG). EcoNI and KpnI restriction sites within this fragment were used to replace the sequence between EcoNI and KpnI in pIdsBB (2). Ligations were transformed into One Shot® OmniMax™ 2 T1^{R}

chemically competent *Escherichia coli* (Thermo Fisher Scientific, Waltham, MA). Oligos were synthesized by Integrated DNA Technologies, Inc. (Coralville, IA), and DNA sequencing was performed by Genewiz (South Plainfield, NJ).

Construction of the vipA mutation

A swarm-capable, spontaneous mutant strain of the BB2000-derived strain lacking full-length E (2) was isolated. This isolate was subjected to phenol-chloroform extractions to isolate genomic DNA (gDNA). gDNA was sheared using a Covaris S 220 (Covaris, Woburn, MA), and a library for whole genome sequencing was prepared using the PrepX ILM DNA Library Kit (WaferGen Biosystems, Fremont, CA) for the Apollo 324 NGS Library Prep System (WaferGen Biosystems, Fremont, CA). The library was sequenced as 100-bp, paired-end reads using an Illumina HiSeq 2500 system (Illumina, San Diego, CA). Reads were aligned to the *P. mirabilis* BB2000 genome (Accession number is CP004022) using Geneious (Biomatters, Auckland, New Zealand). Suppressor-specific polymorphisms were identified by aligning the assembled genome to that of the ancestral strain, BB2000::Δ*ids*. The identified mutation mapped to a gene encoding a *vipA* homolog [T6SS_VipA (PF05591))]. BB2000::Δ*ids,* $vipA_{T95G}$ was then constructed by using the pCS34 forward (CGCGGGCCCGGTATTACCCCATAAATAGTGC) and reverse (CAGCTATATTTGGTTTAACTTAAGGTCTAGAGCGCGC) primers to amplify the *vipA* containing fragment from gDNA of the isolated spontaneous mutant strain. Restriction digestion with ApaI and XbaI was used to introduce this sequence into the suicide vector pKNG101 (34). The resulting vector pCS34 was introduced into mating strain *E. coli* SM10λpir (35) and then mated into BB2000::Δ*ids*. Matings were subjected to antibiotic selection on LSW^- agar (15 μg/ml tetracycline and 25 μg/ml streptomycin). Candidate strains were subjected to sucrose

139 counter-selection as previously described (36). Double-recombinants were confirmed using
140 whole genome sequencing as described above. The Bauer Core Facility at Harvard University
141 performed all genome sequencing.
142
143 **Colony expansion and coswarm assays and viable cell counts**
144 Overnight cultures were normalized to OD_{600} 0.1 and swarm-permissive nutrient plates
145 supplemented with kanamycin were inoculated with one microliter of normalized culture. Plates
146 were incubated at 37 °C for 16 hours, and radii of actively migrating swarms were measured.
147 Additionally, widths of individual swarm rings within the swarm colonies were recorded. For
148 coswarm assays, strains were processed as described and mixed at a ratio of 1:1 where indicated.
149 For viable cell counts after 16 hours, actively migrating swarms were resuspended in six
150 milliliters of LB medium and 20 microliters of the cell suspension were used for a 10-fold
151 dilution series. A total of eight dilutions were prepared for each sample and 10 microliters of
152 each dilution were spotted onto LSW^- agar supplemented with kanamycin.
153 For measuring viable cell counts over time, swarm plates were set up as above. Viable
154 cell counts at time point zero were determined by preparing a 10-fold dilution series of the
155 normalized overnight cultures and spotting 10 microliters of each dilution on LSW^- agar plates
156 supplemented with kanamycin. Viable cell counts at time points two, four, six and eight hours
157 post-inoculation were determined by resuspending swarm colonies in one milliliter LB medium
158 and preparing 10-fold dilution series as described above. 10 microliters of each dilution were
159 spotted onto LSW^- agar supplemented with kanamycin. Dilutions with countable numbers of
160 colonies were used to determine viable cell counts of swarm colonies.
161

Swimming assay

Overnight cultures were normalized to OD_{600} 0.1. An inoculation needle was used to inoculate 0.3% LB nutrient plates supplemented with kanamycin. Plates were incubated at 37 °C for nine hours and diameters of swim colonies were measured.

Measuring generation times

Overnight cultures were normalized to OD_{600} 0.1 in LB medium supplemented with kanamycin. Normalized cultures were grown overnight at 37 °C shaking periodically in a Tecan Infinite® 200 PRO microplate reader (Tecan, Männedorf, Switzerland). Generation times were calculated from logarithmic phase growth measurements.

Trichloroacetic acid precipitations, SDS-PAGE, western blots and LC-MS/MS

Trichloroacetic acid precipitations were performed as previously described (5). Samples were normalized according to OD_{600} of the liquid cultures at collection, separated by gel electrophoresis using 12% Tris-tricine polyacrylamide gels, transferred onto 0.45-μm nitrocellulose membranes, and probed with monoclonal rabbit-α-FLAG (Sigma-Aldrich, St. Louis, MO) and mouse-α-σ^{70} primary antibodies (Thermo Fisher Scientific, Waltham, MA), followed by polyclonal goat-α-rabbit (KPL, Inc., Gaithersburg, MD) and goat-α-mouse secondary antibodies (KPL, Inc., Gaithersburg, MD), respectively. Membranes were developed with the Immun-Star HRP Substrate Kit (Bio-Rad Laboratories, Hercules, CA) and visualized using a Chemidoc (Bio-Rad Laboratories, Hercules, CA). TIFF images were exported and figures were made in Adobe Illustrator (Adobe Systems, San Jose, CA).

To detect secreted proteins in liquid supernatants by mass spectrometry, trichloroacetic acid precipitations were performed as previously described (5). Samples were normalized according to OD_{600}, separated by gel electrophoresis using 12% Tris-tricine polyacrylamide gels and stained with Coomassie Blue. Gel fragments corresponding to molecular weights of approximately 70 to 150 kDa were excised and subjected to liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS). The Taplin Biological Mass Spectrometry Facility (Harvard Medical School, Boston, MA) performed the LC-MS/MS.

Boundary assays

Boundary assays were conducted as previously reported (5). Assays were carried out using swarm-permissive agar plates supplemented with kanamycin.

Phase contrast microscopy

1-mm thick swarm-permissive agar pads supplemented with kanamycin were inoculated from overnight cultures. The agar pads were incubated at 37 °C in a modified humidity chamber. After six hours, the pads were imaged by phase contrast microscopy using a Leica DM5500B (Leica Microsystems, Buffalo Grove, IL) and a CoolSnap HQ^2 cooled CCD camera (Photometrics, Tucson, AZ). MetaMorph version 7.8.0.0 (Molecular Devices, Sunnyvale, CA) was used for image acquisition. Images were acquired every two seconds for 78 seconds. Image stacks were imported into Fiji (ImageJ 1.48s) (37-40) where the image stacks were cropped to show a segment of cells, combined into a single movie from four individual movies, and converted to an .AVI file with a frame rate of five frames per second.

Results

Non-cognate D-E pairs cause restricted swarm colony expansion but not reduced viability or apparent swarmer cell differentiation

Swarming colonies of *P. mirabilis* strain BB2000 carrying mutations in the variable regions (VRs) of the *ids* operon appear unusually small in diameter (31). To investigate effects of Ids-mediated self-recognition on swarm colony expansion, we utilized these *P. mirabilis* strains, which are genetically identical except for the VRs in D and E. The VRs either originated from wild-type strain BB2000 (VR-BB) or from wild-type strain HI4320 (VR-HI). The *ids* operon, including the genes for D and E, was maintained on a low-copy number plasmid under control of the native promoter in the Δ*ids* strain, which is a BB2000-derived strain lacking the chromosomal copy of the *ids* locus (2). This complemented Δ*ids* strain and its derivatives are the standard tools for studying the Ids system (2, 5, 31). We observed that, after 16 hours on swarm-permissive agar, swarm colonies of a strain producing the cognate $D_{VR\text{-}BB}E_{VR\text{-}BB}$ pair (CCS01) expanded further than swarm colonies of strains producing the non-cognate $D_{VR\text{-}HI}E_{VR\text{-}BB}$ (CCS02) or $D_{VR\text{-}BB}E_{VR\text{-}HI}$ (CCS03) pairs (Fig. 1A). In contrast, a strain producing the cognate $D_{VR\text{-}HI}E_{VR\text{-}HI}$ pair (CCS04) showed recovered swarm expansion (Fig. 1A). Differences in colony expansion persisted even after 24 hours (Fig. 1B). Thus, swarm colony expansion was restricted when non-cognate D and E proteins are present.

Whether D and E contained cognate or non-cognate variable regions, however, had no measurable effect on the number of swarm rings per colony (Fig. 1A), on growth on surfaces (Fig. 1C, Fig. 2A), or growth in liquid (Fig. 2B), suggesting that growth and swarm-related developmental cycles were not impaired. Marginal differences in colony expansion during

swimming in low-percentage agar were observed between these strains (Fig. 1D), and individual cells of all four strains were capable of differentiating into elongated, actively moving swarmer cells (Movie 1). Therefore, non-cognate D-E pairs do not appear to inhibit cell viability, swimming motility, or swarm colony development; nevertheless, macroscopic swarm colony expansion was impaired. We reasoned that this stark phenotype could be used to address the outstanding question of how the Ids system communicates identity information between cells within a colony.

D communicates identity between neighboring cells

There are two prevailing mechanistic models for where the causative *in vivo* interactions between D and E may occur. D-E binding could happen between neighboring cells (Fig. 3A) or within a single cell (Fig. 3B). To distinguish between these models, we first examined whether D export is necessary for its function. We used a Δ*ids*-derived strain deficient in T6SS-mediated transport (CCS05). CCS05 carries a mutation in *vipA*; the encoded protein, VipA [T6SS_VipA (PF05591)], is essential for export of T6SS-related factors (11, 42, 43). To confirm loss of T6SS-mediated transport, IdsA [T6SS_Hcp (PF05638)] carrying a C-terminal FLAG epitope tag (5) was introduced into CCS05. Export of Hcp homologs, such as IdsA, is a hallmark of T6SS-dependent protein transport and has often been used to evaluate T6SS activity (5, 8, 9, 11, 26, 44). Further, the export of D, as well as IdsB, is dependent on a functional T6SS and has been correlated with IdsA export (5). The export of IdsA-FLAG from the CCS05-derived strain was markedly decreased as compared to that from the otherwise genetically equivalent strain expressing wild-type *vipA* (Fig. 3C). We then analyzed supernatants isolated from these strains by LC-MS/MS and found that peptides corresponding to IdsB and D were absent in the CCS05-

253 derived strain expressing mutant *vipA* as compared to the Δ*ids*-derived strain expressing wild-
254 type *vipA* (Table 2). These results indicate that CCS05 is deficient in T6SS-mediated export,
255 including the loss of IdsA, IdsB, and D transport.
256 We utilized CCS05-derived strains expressing different combinations of D and E variants,
257 as described in Figure 1, as indicator strains to determine whether D is received from a
258 neighboring cell. We determined boundary formation phenotypes of these CCS05-derived strains
259 when swarmed against Δ*ids*-derived (export-active donor) strains that produced D and E proteins
260 either from strain BB2000 ($D_{VR\text{-}BB}E_{VR\text{-}BB}$) or from strain HI4320 ($D_{HI}E_{HI}$). These two export-
261 active strains form a boundary against each other and are non-self (31). Swarming populations of
262 the CCS05-derived strains producing $E_{VR\text{-}BB}$ (and either $D_{VR\text{-}BB}$ or $D_{VR\text{-}HI}$) merged with the donor
263 strain producing $D_{VR\text{-}BB}E_{VR\text{-}BB}$ and not with the donor strain producing $D_{HI}E_{HI}$ (Fig. 3D).
264 Conversely, CCS05-derived strains producing $E_{VR\text{-}HI}$ (and either $D_{VR\text{-}BB}$ or $D_{VR\text{-}HI}$) merged with
265 the donor strain producing $D_{HI}E_{HI}$ (Fig. 3D). In all cases, the D variant produced by the CCS05-
266 derived, export-inactive strain did not affect the outcome (Fig. 3D). Thus the identities of the D
267 variant in the donor strain and of the E variant in the export-inactive CCS05-derived strain
268 correlated with whether populations merged or formed a boundary.
269 Given these data, the observed impairment in swarm colony expansion of CCS02 and
270 CCS03, which are the strains producing non-cognate D and E proteins (Fig. 1A), could be
271 explained by the presence of unbound D in recipient cells (Fig. 3A). If so, then a similar defect
272 would be expected for strains lacking E since, in a clonal population, every cell could export as
273 well as receive D and would have no E to bind it. We constructed a Δ*ids*-derived strain
274 complemented with an *ids* operon that lacks the gene encoding E (CCS06) to test this hypothesis.
275 CCS06 swarms displayed colony expansion similar to that of CCS02 and CCS03 (Fig. 4A).

CCS06 did not exhibit defects in swarm rings per colony (Fig. 4A), growth on surfaces (Fig. 2A), or growth in liquid (Fig. 2B). Therefore, the presence of unbound D indeed impaired swarm colony expansion.

The question remained, however, whether D exchange between cells is crucial for this inhibition of swarm colony expansion or whether unbound, self-produced D could also affect this self-recognition behavior. Therefore, we examined the swarm colony expansion of export-inactive, CCS05-derived cells lacking E. In this strain, cells contain self-produced D but cannot export D, i.e., cells do not contain transferred D. This strain exhibited a rescued swarm colony expansion phenotype (Fig. 4A). Together these results support the hypothesis that D is exported and that it is transferred between cells (Fig. 3A). Moreover, transferred, unbound D in recipient cells, rather than self-produced D, appears to impair swarm colony expansion.

Interactions between transferred D and resident E impact swarm colony expansion

We hypothesized that the transfer of D might be sufficient to induce impaired swarm colony expansion. We interrogated this hypothesis by examining the swarm colony expansion of 1:1 mixtures of two strains, resulting in coswarms. Strain CCS06 (lacking E) was coswarmed with the nearly isogenic CCS05-derived recipient strain lacking both E and a functional T6SS. We observed a 1.75-fold decrease in expansion of the coswarm colony as compared to that of a monoculture swarm of the recipient strain (Fig. 4A), indicating that transfer of D to recipient cells restricted swarm colony expansion.

We further hypothesized that transfer of D and its resulting binding state with E in the recipient cell determines whether swarm colony expansion is restricted or not. Therefore, we used CCS06 as a donor of $D_{VR\text{-}BB}$ in 1:1 mixtures with CCS05-derived (export-inactive recipient)

299 strains that produced either $E_{VR\text{-}BB}$, which binds $D_{VR\text{-}BB}$, or $E_{VR\text{-}HI}$, which cannot bind $D_{VR\text{-}BB}$. All
300 coswarms were compared to monoculture swarms of the recipient strain. In coswarms of CCS06
301 with the recipient strain producing $D_{VR\text{-}BB}$ and $E_{VR\text{-}HI}$, a 3.12-fold reduction was observed (Fig.
302 4). Likewise, a coswarm of CCS06 with the recipient strain producing $D_{VR\text{-}HI}$ and $E_{VR\text{-}HI}$ resulted
303 in a 3.35-fold reduction in colony expansion (Fig. 4). By contrast, mixing CCS06 with the
304 recipient strain producing $D_{VR\text{-}BB}$ and $E_{VR\text{-}BB}$ resulted in marginal reduction of swarm colony
305 expansion (Fig. 4). In sum, only marginal restriction appeared when the E variant in the recipient
306 strain is capable of binding $D_{VR\text{-}BB}$ from the donor strain. However, we observed a ~ 2 – 3 fold
307 restriction in swarm colony expansion when E in the recipient strain is non-cognate to $D_{VR\text{-}BB}$.
308 Thus communication of D from a donor to a recipient cell causes restricted swarm colony
309 expansion. Alleviation of this swarm restriction can be achieved by the presence of a cognate E
310 in the recipient cell.
311 During the course of observing monoclonal swarms, we unexpectedly noticed that the
312 production of E in recipient strains, regardless of whether a cognate D was produced, resulted in
313 a marked decrease of colony expansion (average = 2.3-fold) as compared to that for an otherwise
314 identical strain that lacked E (Fig. 4A). These results raise the possibility that independently of D,
315 E itself contributes to repression of swarm colony expansion.
316
317 Discussion
318 Here, we have addressed unresolved questions regarding the communication of Ids proteins
319 within a colony of swarming *P. mirabilis*. We have shown that the self-identity protein D is
320 communicated from one cell to another. We have also presented evidence that D from donor
321 cells likely interacts with E in recipient cells; lack of this interaction negatively impacts swarm

colony expansion, but not viability. Therefore, D might represent a class of non-lethal T6SS effector proteins.

Based on the prominent T6SS models for effector/inhibitor pairs, it was expected that unbound D, whether in donor or recipient cells, should suffice to act as an effector; however this was not strictly observed since unbound D was only active in recipient cells. D might not be in a folded or active state in the donor cell. Even more surprisingly, the presence of E in export-impaired cells appeared to have an inhibitory effect on colony expansion (Fig. 4A). Together our observations suggest that D and E regulate swarm colony expansion; the specific molecular mechanisms, however, remain to be determined.

It is a bit perplexing that D is indeed communicated between cells in a T6SS-dependent manner as D is over 100 kDa in size and contains two predicted transmembrane segments (31). Many T6SS-exported effectors are under 50 kDa, and the inner Hcp tube comprising the channel of many T6SSs has a width of 40 Å in multiple bacteria (8, 45-48). In fact, a variety of T6SS effectors bind to the inside of the Hcp tube allowing them to be exported (44). The size of the *P. mirabilis* T6SS pore has not been directly measured. Given D's large size, D might be communicated via the T6SS by an alternative mechanism. For example, D might be exported out of the donor cell in an unfolded state and then fold into its active state before or after being received by the recipient cell. This would be consistent with the observation that D transfer is required for its activity (Fig. 4A). Clearly, the macromolecular states of D before, during, and after transfer remain to be resolved to explain this transfer.

Microbial communities frequently exhibit cell-to-cell communication, in many cases involving the exchange of information about kin group identity. Self versus non-self recognition allows that certain group behaviors primarily occur between closely related individuals and/or

exclude non-kin cells from shared resources. Many of the mechanisms for the exchange of kin group identity can be distinguished based on their contact-dependency or effects on viability. Quorum sensing by which groups of bacteria can roughly assess cell population density is an example of contact-independent recognition. In this case, kin group identity information is encoded by the molecular structure of the quorum sensing molecule and its ability to bind its protein receptor (49-52). However, as quorum sensing molecules are often diffusible across membranes (53, 54), recognition events do not require physical contact between cells and can occur over greater spatial distances than contact-dependent mechanisms.

By contrast, contact-dependent interactions are local. These recognition events usually require cell-to-cell contact and can involve lethal attacks on non-kin members of the community. For example, contact-dependent killing mechanisms have been described for antagonistic interactions between species and even genera, e.g., T6SS-associated killing (8-10, 13, 14, 16-20, 27, 28), and within a species, e.g., contact-dependent inhibition (CDI) (55-62). From a competition perspective this could be beneficial; if susceptible competitor cells are inhibited, fewer cells will compete for resources like nutrients. However, the existence of contact-dependent recognition that does not involve killing, such as demonstrated here for the self-identity proteins D and E, suggests that there likely is a fitness and competitive advantage to recognizing cells of the same kin group. For sibling cells of the bacterium *Myxococcus xanthus*, fusion of outer membranes, which is mediated by a recognition protein, can contribute to overall increased colony fitness (63). For *P. mirabilis*, swarm expansion of the colony involves intimate interactions between individual cells (64), and so cooperation might be essential for long-range motility. One purpose of the Ids system, and specifically of the self-identity proteins, D and E, might be to restrict cooperative motility behavior to kin cells. As such, the transfer of D and its

subsequent interactions with E may represent an additional form of cell-cell communication within a bacterial population.

Acknowledgements

We would like to thank Lia Cardarelli, Richard Losick, and members of the Gibbs Lab for advice and thoughtful discussions. Funding from the David and Lucile Packard Foundation, the George W. Merck Fund, and Harvard University supported this research.

References

1. **Budding AE, Ingham CJ, Bitter W, Vandenbroucke-Grauls CM, Schneeberger PM.** 2009. The Dienes phenomenon: competition and territoriality in swarming *Proteus mirabilis*. J Bacteriol **191:**3892-3900.

2. **Gibbs KA, Urbanowski ML, Greenberg EP.** 2008. Genetic determinants of self identity and social recognition in bacteria. Science **321:**256-259.

3. **Senior BW.** 1977. The Dienes phenomenon: identification of the determinants of compatibility. J Gen Microbiol **102:**235-244.

4. **Dienes L.** 1947. Further observations on the reproduction of bacilli from large bodies in *Proteus* cultures. Proc Soc Exp Biol Med **66:**97-98.

5. **Wenren LM, Sullivan NL, Cardarelli L, Septer AN, Gibbs KA.** 2013. Two independent pathways for self-recognition in *Proteus mirabilis* are linked by type VI-dependent export. mBio **4**.

6. **Gibbs KA, Wenren LM, Greenberg EP.** 2011. Identity gene expression in *Proteus mirabilis*. J Bacteriol **193:**3286-3292.

7. **Alteri CJ, Himpsl SD, Pickens SR, Lindner JR, Zora JS, Miller JE, Arno PD, Straight SW, Mobley HL.** 2013. Multicellular bacteria deploy the type VI secretion system to preemptively strike neighboring cells. PLoS Pathog **9:**e1003608.

8. **Mougous JD, Cuff ME, Raunser S, Shen A, Zhou M, Gifford CA, Goodman AL, Joachimiak G, Ordonez CL, Lory S, Walz T, Joachimiak A, Mekalanos JJ.** 2006. A virulence locus of *Pseudomonas aeruginosa* encodes a protein secretion apparatus. Science **312:**1526-1530.

9. **Pukatzki S, Ma AT, Sturtevant D, Krastins B, Sarracino D, Nelson WC, Heidelberg JF, Mekalanos JJ.** 2006. Identification of a conserved bacterial protein secretion system in *Vibrio cholerae* using the *Dictyostelium* host model system. Proc Natl Acad Sci U S A **103:**1528-1533.

10. **Pukatzki S, Ma AT, Revel AT, Sturtevant D, Mekalanos JJ.** 2007. Type VI secretion system translocates a phage tail spike-like protein into target cells where it cross-links actin. Proc Natl Acad Sci U S A **104:**15508-15513.

11. **Basler M, Pilhofer M, Henderson GP, Jensen GJ, Mekalanos JJ.** 2012. Type VI secretion requires a dynamic contractile phage tail-like structure. Nature **483:**182-186.

12. **Basler M, Mekalanos JJ.** 2012. Type 6 secretion dynamics within and between bacterial cells. Science **337:**815.

13. **Basler M, Ho BT, Mekalanos JJ.** 2013. Tit-for-tat: type VI secretion system counterattack during bacterial cell-cell interactions. Cell **152:**884-894.

14. **Shneider MM, Buth SA, Ho BT, Basler M, Mekalanos JJ, Leiman PG.** 2013. PAAR-repeat proteins sharpen and diversify the type VI secretion system spike. Nature **500:**350-353.

15. **Hood RD, Singh P, Hsu F, Guvener T, Carl MA, Trinidad RR, Silverman JM, Ohlson BB, Hicks KG, Plemel RL, Li M, Schwarz S, Wang WY, Merz AJ, Goodlett DR, Mougous JD.** 2010. A type VI secretion system of *Pseudomonas aeruginosa* targets a toxin to bacteria. Cell Host Microbe **7:**25-37.

16. **Russell AB, Hood RD, Bui NK, LeRoux M, Vollmer W, Mougous JD.** 2011. Type VI secretion delivers bacteriolytic effectors to target cells. Nature **475:**343-347.

17. **Russell AB, LeRoux M, Hathazi K, Agnello DM, Ishikawa T, Wiggins PA, Wai SN, Mougous JD.** 2013. Diverse type VI secretion phospholipases are functionally plastic antibacterial effectors. Nature **496:**508-512.

18. **Whitney JC, Chou S, Russell AB, Biboy J, Gardiner TE, Ferrin MA, Brittnacher M, Vollmer W, Mougous JD.** 2013. Identification, structure, and function of a novel type VI secretion peptidoglycan glycoside hydrolase effector-immunity pair. J Biol Chem **288:**26616-26624.

19. **MacIntyre DL, Miyata ST, Kitaoka M, Pukatzki S.** 2010. The *Vibrio cholerae* type VI secretion system displays antimicrobial properties. Proc Natl Acad Sci U S A **107:**19520-19524.

20. **Miyata ST, Kitaoka M, Brooks TM, McAuley SB, Pukatzki S.** 2011. *Vibrio cholerae* requires the type VI secretion system virulence factor VasX to kill *Dictyostelium discoideum*. Infect Immun **79:**2941-2949.

21. **Brooks TM, Unterweger D, Bachmann V, Kostiuk B, Pukatzki S.** 2013. Lytic activity of the *Vibrio cholerae* type VI secretion toxin VgrG-3 is inhibited by the antitoxin TsaB. J Biol Chem **288:**7618-7625.

22. **Unterweger D, Miyata ST, Bachmann V, Brooks TM, Mullins T, Kostiuk B, Provenzano D, Pukatzki S.** 2014. The *Vibrio cholerae* type VI secretion system employs diverse effector modules for intraspecific competition. Nat Commun **5:**3549.

23. **Durand E, Derrez E, Audoly G, Spinelli S, Ortiz-Lombardia M, Raoult D, Cascales E, Cambillau C.** 2012. Crystal structure of the VgrG1 actin cross-linking domain of the *Vibrio cholerae* type VI secretion system. J Biol Chem **287:**38190-38199.

24. **Brunet YR, Espinosa L, Harchouni S, Mignot T, Cascales E.** 2013. Imaging type VI secretion-mediated bacterial killing. Cell Rep **3:**36-41.

25. **Durand E, Nguyen VS, Zoued A, Logger L, Pehau-Arnaudet G, Aschtgen MS, Spinelli S, Desmyter A, Bardiaux B, Dujeancourt A, Roussel A, Cambillau C, Cascales E, Fronzes R.** 2015. Biogenesis and structure of a type VI secretion membrane core complex. Nature **523:**555-560.

26. **Hachani A, Lossi NS, Hamilton A, Jones C, Bleves S, Albesa-Jove D, Filloux A.** 2011. Type VI secretion system in *Pseudomonas aeruginosa*: secretion and multimerization of VgrG proteins. J Biol Chem **286:**12317-12327.

27. **Hachani A, Allsopp LP, Oduko Y, Filloux A.** 2014. The VgrG proteins are "a la carte" delivery systems for bacterial type VI effectors. J Biol Chem **289:**17872-17884.

28. **Ma LS, Hachani A, Lin JS, Filloux A, Lai EM.** 2014. *Agrobacterium tumefaciens* deploys a superfamily of type VI secretion DNase effectors as weapons for interbacterial competition in planta. Cell Host Microbe **16:**94-104.

29. **Li M, Le Trong I, Carl MA, Larson ET, Chou S, De Leon JA, Dove SL, Stenkamp RE, Mougous JD.** 2012. Structural basis for type VI secretion effector recognition by a cognate immunity protein. PLoS Pathog **8:**e1002613.

30. **Miyata ST, Unterweger D, Rudko SP, Pukatzki S.** 2013. Dual expression profile of type VI secretion system immunity genes protects pandemic *Vibrio cholerae*. PLoS Pathog **9:**e1003752.

31. **Cardarelli L, Saak C, Gibbs KA.** 2015. Two proteins form a heteromeric bacterial self-recognition complex in which variable subdomains determine allele-restricted binding. mBio **6:**e00251.

32. **Salomon D, Kinch LN, Trudgian DC, Guo X, Klimko JA, Grishin NV, Mirzaei H, Orth K.** 2014. Marker for type VI secretion system effectors. Proc Natl Acad Sci U S A **111:**9271-9276.

33. **Belas R, Erskine D, Flaherty D.** 1991. Transposon mutagenesis in *Proteus mirabilis*. J Bacteriol **173:**6289-6293.

34. **Kaniga K, Delor I, Cornelis GR.** 1991. A wide-host-range suicide vector for improving reverse genetics in gram-negative bacteria: inactivation of the *blaA* gene of *Yersinia enterocolitica*. Gene **109:**137-141.

35. **de Lorenzo V, Herrero M, Jakubzik U, Timmis KN.** 1990. Mini-Tn5 transposon derivatives for insertion mutagenesis, promoter probing, and chromosomal insertion of cloned DNA in gram-negative eubacteria. J Bacteriol **172:**6568-6572.

36. **Sturgill GM, Siddiqui S, Ding X, Pecora ND, Rather PN.** 2002. Isolation of *lacZ* fusions to *Proteus mirabilis* genes regulated by intercellular signaling: potential role for the sugar phosphotransferase (Pts) system in regulation. FEMS Microbiol Lett **217:**43-50.

37. **Schneider CA, Rasband WS, Eliceiri KW.** 2012. NIH Image to ImageJ: 25 years of image analysis. Nat Methods **9:**671-675.

38. **Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B, Tinevez JY, White DJ, Hartenstein V, Eliceiri K, Tomancak P, Cardona A.** 2012. Fiji: an open-source platform for biological-image analysis. Nat Methods **9:**676-682.

39. **Schindelin J, Rueden CT, Hiner MC, Eliceiri KW.** 2015. The ImageJ ecosystem: An open platform for biomedical image analysis. Mol Reprod Dev **82:**518-529.

40. **Pietzsch T, Preibisch S, Tomancak P, Saalfeld S.** 2012. ImgLib2--generic image processing in Java. Bioinformatics **28:**3009-3011.

41. **Simon R, Priefer U, Pühler A.** 1983. A broad host range mobilization system for *in vivo* genetic engineering: transposon mutagenesis in gram negative bacteria. Nat Biotechnology **1:**784-791.

42. **Bonemann G, Pietrosiuk A, Diemand A, Zentgraf H, Mogk A.** 2009. Remodelling of VipA/VipB tubules by ClpV-mediated threading is crucial for type VI protein secretion. Embo J **28:**315-325.

43. **Kapitein N, Bonemann G, Pietrosiuk A, Seyffer F, Hausser I, Locker JK, Mogk A.** 2013. ClpV recycles VipA/VipB tubules and prevents non-productive tubule formation to ensure efficient type VI protein secretion. Mol Microbiol **87:**1013-1028.

44. **Silverman JM, Agnello DM, Zheng H, Andrews BT, Li M, Catalano CE, Gonen T, Mougous JD.** 2013. Haemolysin coregulated protein is an exported receptor and chaperone of type VI secretion substrates. Mol Cell **51:**584-593.

45. **Ruiz FM, Santillana E, Spinola-Amilibia M, Torreira E, Culebras E, Romero A.** 2015. Correction: Crystal Structure of Hcp from *Acinetobacter baumannii*: A Component of the Type VI Secretion System. PLoS One **10:**e0136978.

46. **Osipiuk J, Xu X, Cui H, Savchenko A, Edwards A, Joachimiak A.** 2011. Crystal structure of secretory protein Hcp3 from *Pseudomonas aeruginosa*. J Struct Funct Genomics **12:**21-26.

47. **Douzi B, Spinelli S, Blangy S, Roussel A, Durand E, Brunet YR, Cascales E, Cambillau C.** 2014. Crystal structure and self-interaction of the type VI secretion tail-tube protein from enteroaggregative *Escherichia coli*. PLoS One **9:**e86918.

48. **Ruiz FM, Santillana E, Spinola-Amilibia M, Torreira E, Culebras E, Romero A.** 2015. Crystal Structure of Hcp from *Acinetobacter baumannii*: A Component of the Type VI Secretion System. PLoS One **10:**e0129691.

49. **Gray KM, Passador L, Iglewski BH, Greenberg EP.** 1994. Interchangeability and specificity of components from the quorum-sensing regulatory systems of *Vibrio fischeri* and *Pseudomonas aeruginosa*. J Bacteriol **176:**3076-3080.

50. **Waters CM, Bassler BL.** 2005. Quorum sensing: cell-to-cell communication in bacteria. Annu Rev Cell Dev Biol **21:**319-346.

51. **Vannini A, Volpari C, Gargioli C, Muraglia E, Cortese R, De Francesco R, Neddermann P, Marco SD.** 2002. The crystal structure of the quorum sensing protein TraR bound to its autoinducer and target DNA. EMBO J **21:**4393-4401.

52. **Zhang RG, Pappas KM, Brace JL, Miller PC, Oulmassov T, Molyneaux JM, Anderson JC, Bashkin JK, Winans SC, Joachimiak A.** 2002. Structure of a bacterial quorum-sensing transcription factor complexed with pheromone and DNA. Nature **417:**971-974.

53. **Kaplan HB, Greenberg EP.** 1985. Diffusion of autoinducer is involved in regulation of the *Vibrio-Fischeri* luminescence system. J Bacteriol **163:**1210-1214.

54. **Pearson JP, Van Delden C, Iglewski BH.** 1998. Active efflux and diffusion are involved in transport of *Pseudomonas aeruginosa* cell-to-cell signals. J Bacteriol **181:**1203-1210.

55. **Aoki SK, Pamma R, Hernday AD, Bickham JE, Braaten BA, Low DA.** 2005. Contact-dependent inhibition of growth in *Escherichia coli*. *Science* **309:**1245-1248.

56. **Aoki SK, Malinverni JC, Jacoby K, Thomas B, Pamma R, Trinh BN, Remers S, Webb J, Braaten BA, Silhavy TJ, Low DA.** 2008. Contact-dependent growth inhibition

requires the essential outer membrane protein BamA (YaeT) as the receptor and the inner membrane transport protein AcrB. Mol Microbiol **70:**323-340.

57. **Aoki SK, Diner EJ, de Roodenbeke CT, Burgess BR, Poole SJ, Braaten BA, Jones AM, Webb JS, Hayes CS, Cotter PA, Low DA.** 2010. A widespread family of polymorphic contact-dependent toxin delivery systems in bacteria. Nature **468:**439-442.

58. **Poole SJ, Diner EJ, Aoki SK, Braaten BA, t'Kint de Roodenbeke C, Low DA, Hayes CS.** 2011. Identification of functional toxin/immunity genes linked to contact-dependent growth inhibition (CDI) and rearrangement hotspot (Rhs) systems. PLoS Genet **7:**e1002217.

59. **Diner EJ, Beck CM, Webb JS, Low DA, Hayes CS.** 2012. Identification of a target cell permissive factor required for contact-dependent growth inhibition (CDI). Genes Dev **26:**515-525.

60. **Ruhe ZC, Wallace AB, Low DA, Hayes CS.** 2013. Receptor polymorphism restricts contact-dependent growth inhibition to members of the same species. mBio **4**.

61. **Anderson MS, Garcia EC, Cotter PA.** 2012. The *Burkholderia bcpAIOB* genes define unique classes of two-partner secretion and contact dependent growth inhibition systems. PLoS Genet **8:**e1002877.

62. **Anderson MS, Garcia EC, Cotter PA.** 2014. Kind discrimination and competitive exclusion mediated by contact-dependent growth inhibition systems shape biofilm community structure. PLoS Pathog **10:**e1004076.

63. **Vassallo C, Pathak DT, Cao P, Zuckerman DM, Hoiczyk E, Wall D.** 2015. Cell rejuvenation and social behaviors promoted by LPS exchange in *myxobacteria*. Proc Natl Acad Sci U S A **112:**E2939-2946.

64. **Jones BV, Young R, Mahenthiralingam E, Stickler DJ.** 2004. Ultrastructure of *Proteus mirabilis* swarmer cell rafts and role of swarming in catheter-associated urinary tract infection. Infect Immun **72:**3941-3950.

Figure legends

Figure 1. The binding states of D and E regulate swarm colony expansion.

(A) Colony expansion of monoclonal *P. mirabilis* swarms after 16 hours on swarm-permissive agar surfaces. Variable region (VR) identities of the produced D and E variants are indicated for each strain. Widths of individual swarm rings within a swarm colony are marked by different shades. N = 16, error bars show standard deviations of individual swarm ring widths.

(B) Representative pictures of each strain from Fig. 1A were taken 24 hours after inoculation.

(C) Viable cells per monoclonal swarm colony after 16 hours on swarm-permissive agar surfaces. Strain descriptions are found in Fig. 1D. N = 12.

(D) Diameters of monoclonal colonies in 0.3% LB medium after 9 hours. N = 6.

Figure 2. Viability on surfaces and generation times in liquid are unaltered when D and E are noncognate.

(A) Viable cells per swarm colony over time on swarm-permissive agar surfaces. D and E variants produced by the different strains are indicated in Fig. 2B. N = 4, error bars show standard deviations.

(B) Generation times during logarithmic growth in liquid medium. N = 6.

Figure 3. D is communicated between cells.

(A, B) Competing mechanistic models for the mode of D-E interactions. **(A)** Intercellular, T6SS-dependent (grey arrow) communication of D from one cell (double-walled oval) to a neighboring cell. Binding to E in the recipient cell allows for swarm colony expansion to proceed, while lack thereof impairs colony expansion. This communication is bidirectional if both cells have a functional T6SS. **(B)** Swarm colony expansion depends on the binding states of the D and E variants produced within an individual cell.

(C) Supernatants of strains carrying either the empty vector pKG101 (6) or pLW101, which produces IdsA [T6SS_Hcp (PF05638)] with a C-terminal FLAG tag (5), were subjected to trichloroacetic acid precipitations. Whole cell extracts were obtained as well. All samples were analyzed using Western blot analysis. The BB2000-derived *vipA::Tn5* strain contains a chromosomal transposon insertion in the gene encoding VipA [T6SS_VipA (PF05591)] (5). Blots were probed with antibodies against FLAG to detect IdsA-FLAG and against σ^{70} as a cell lysis control.

(D) Swarm-permissive agar surfaces were inoculated with Δ*ids*-derived (export-active, donor) strains on the left side and CCS05-derived (export-inactive, recipient) strains on the right side. Each strain produces the indicated D and E variants. Variable region exchanges from BB2000 (BB) to HI4320 (HI) are indicated with the prefix "VR-". D_{HI} and E_{HI} are D and E variants derived completely from HI4320. Green rectangles outline combinations of swarms that merged. Arrowheads indicate where opposing swarm colonies intersect.

Figure 4. Unbound D in a recipient cell impairs swarm colony expansion.

(A) Colony expansion as described in Fig. 1A. Strains were inoculated either as monoswarms (export-active CCS06 donor or export-inactive CCS05-derived recipients) or as coswarms (CCS06 and CCS05-derivatives) at a 1:1 ratio. D and E variants produced by strains derived from CCS05 are indicated. CCS06 lacks E, but produces $D_{VR\text{-}BB}$. N = 3 for mono- and coswarms of $D_{VR\text{-}BB}E_{VR\text{-}HI}$ and $D_{VR\text{-}HI}E_{VR\text{-}HI}$, n = 6 for all others. Error bars show standard deviations for each swarm ring width. Fold changes of total colony expansion between mono- and coswarms are indicated.

(B) Representative pictures of mono- and coswarms were taken 24 hours after inoculation.

(C) Models depict intercellular, T6SS-dependent (grey arrow) communication of $D_{VR\text{-}BB}$ from an export-active cell (CCS06, grey) to its neighboring cell, whether export-active (grey) or export-inactive (CCS05-derivative, white). For export-inactive cells, only the resident E is depicted, whereas the resident D is omitted from the picture.

613 Tables

614 **Table 1. Strains used in this study.**

Strain	Name in this study	Description	Source
Proteus mirabilis			
BB2000		Produces the cognate $D_{VR\text{-}BB}E_{VR\text{-}BB}$ pair from a single allele	(33)
BB2000::Δ*ids*	Δ*ids*	Δ*ids::Tn-Cm*(R) producing neither D nor E	(2)
Δ*ids* c. pIdsBB	CCS01	Δ*ids::Tn-Cm*(R) producing the cognate $D_{VR\text{-}BB}E_{VR\text{-}BB}$ pair	(2)
Δ*ids* c. pIdsBB-$D_{VR\text{-}HI}$-$E_{VR\text{-}BB}$	CCS02	Δ*ids::Tn-Cm*(R) producing the non-cognate $D_{VR\text{-}HI}E_{VR\text{-}BB}$ pair	(31)
Δ*ids* c. pIdsBB-$D_{VR\text{-}BB}$-$E_{VR\text{-}HI}$	CCS03	Δ*ids::Tn-Cm*(R) producing the non-cognate $D_{VR\text{-}BB}E_{VR\text{-}HI}$ pair	(31)
Δ*ids* c. pIdsBB-$D_{VR\text{-}HI}$-$E_{VR\text{-}HI}$	CCS04	Δ*ids::Tn-Cm*(R) producing the cognate $D_{VR\text{-}HI}E_{VR\text{-}HI}$ pair	(31)
Δ*ids* c. pIdsBB-E-mt		Δ*ids::Tn-Cm*(R) producing $D_{VR\text{-}BB}$ and not E	(2)
Δ*ids* c. pKG101		Δ*ids::Tn-Cm*(R) carrying plasmid pKG101, which encodes promoterless *gfp*	(6)

Δ*ids* c. pLW101		Δ*ids::Tn-Cm*(R) carrying a derivative of pIdsBB in which a FLAG epitope (N-DYKDDDDK-C) was inserted immediately before the *idsA* stop codon	(5)
vipA::Tn5 c. pLW101		*vipA::Tn5-Cm*(R) carrying pLW101. *vipA::Tn5-Cm*(R) is strain *tssA** in (5)	(5)
Δ*ids, vipA*$_{T95G}$	CCS05	Δ*ids::Tn-Cm*(R), *vipA*$_{T95G}$ is deficient in T6SS-mediated transport	This study
CCS05 c. pLW101		Δ*ids::Tn-Cm*(R), *vipA*$_{T95G}$ carrying pLW101	This study
Δ*ids* c. pIdsHI		Δ*ids::Tn-Cm*(R) producing the cognate $D_{HI}E_{HI}$ pair	(31)
CCS05 c. pIdsBB		Δ*ids::Tn-Cm*(R), *vipA*$_{T95G}$ producing the cognate $D_{VR\text{-}BB}E_{VR\text{-}BB}$ pair	This study
CCS05 c. pIdsBB-$D_{VR\text{-}HI}$-$E_{VR\text{-}BB}$		Δ*ids::Tn-Cm*(R), *vipA*$_{T95G}$ producing the noncognate $D_{VR\text{-}HI}E_{VR\text{-}BB}$ pair	This study
CCS05 c. pIdsBB-$D_{VR\text{-}BB}$-$E_{VR\text{-}HI}$		Δ*ids::Tn-Cm*(R), *vipA*$_{T95G}$ producing the noncognate $D_{VR\text{-}BB}E_{VR\text{-}HI}$ pair	This study
CCS05 c. pIdsBB-$D_{VR\text{-}HI}$-$E_{VR\text{-}HI}$		Δ*ids::Tn-Cm*(R), *vipA*$_{T95G}$ producing the cognate $D_{VR\text{-}HI}E_{VR\text{-}HI}$ pair	This study
Δ*ids* c. pIdsBB-ΔE	CCS06	Δ*ids::Tn-Cm*(R) producing $D_{VR\text{-}BB}$ and not E	This study

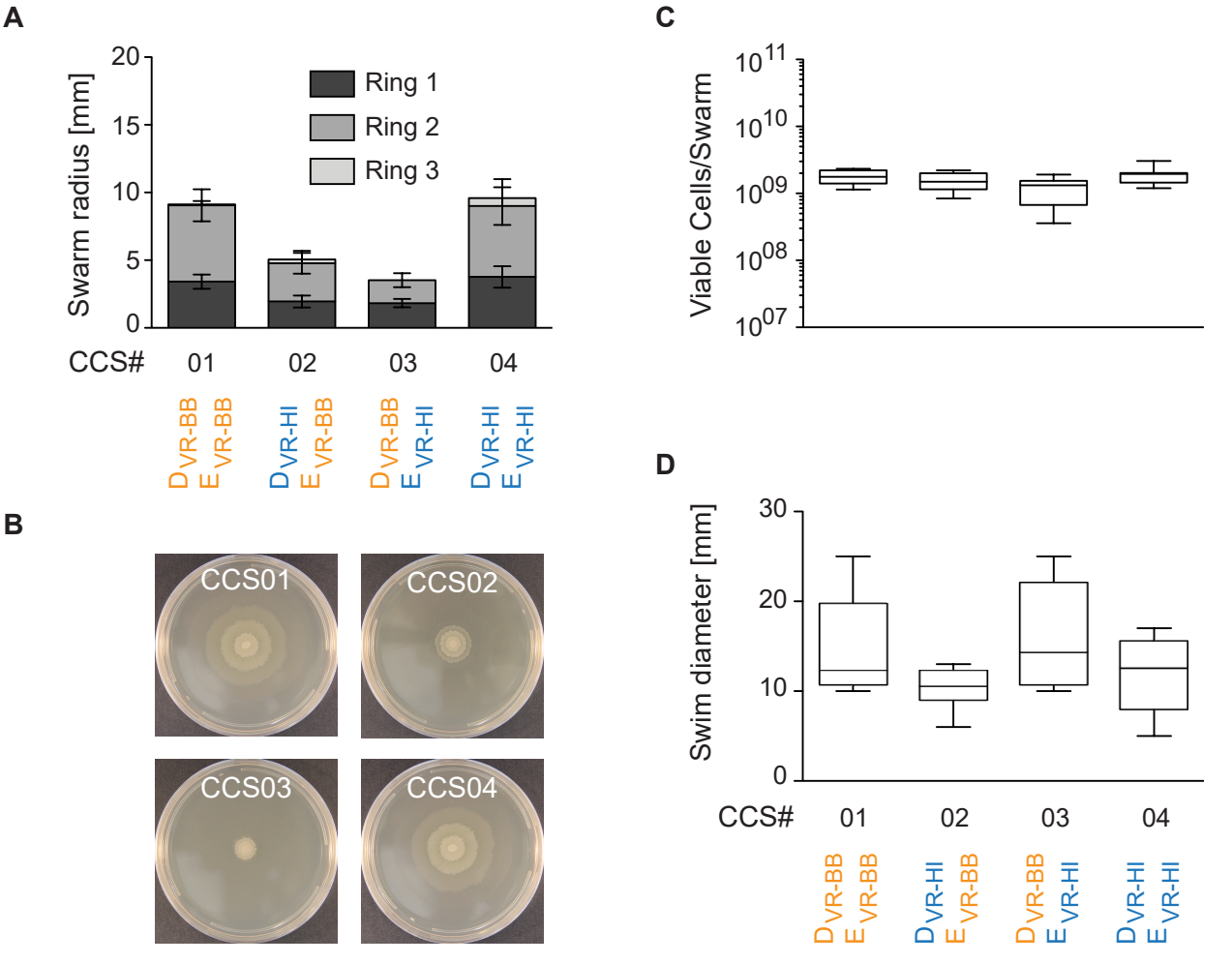
CCS05 c. pIdsBB-ΔE		Δ*ids::Tn-Cm*(R)*, vipA*$_{T95G}$ producing $D_{VR\text{-}BB}$ and not E	This study
Escherichia coli			
S17λpir		Mating strain for moving plasmids from *E. coli* into *P. mirabilis*	(41)
SM10λpir		Mating strain for moving suicide vector pKNG101 (34) into *P. mirabilis*	(35)

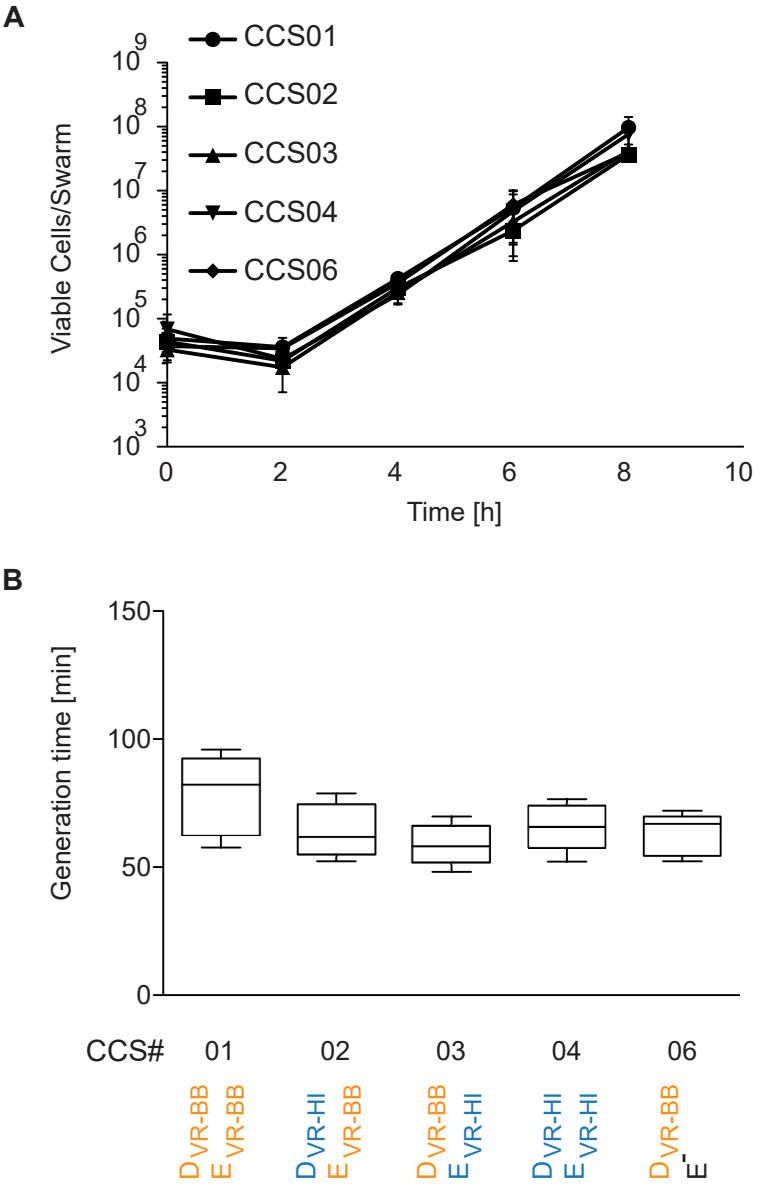
615

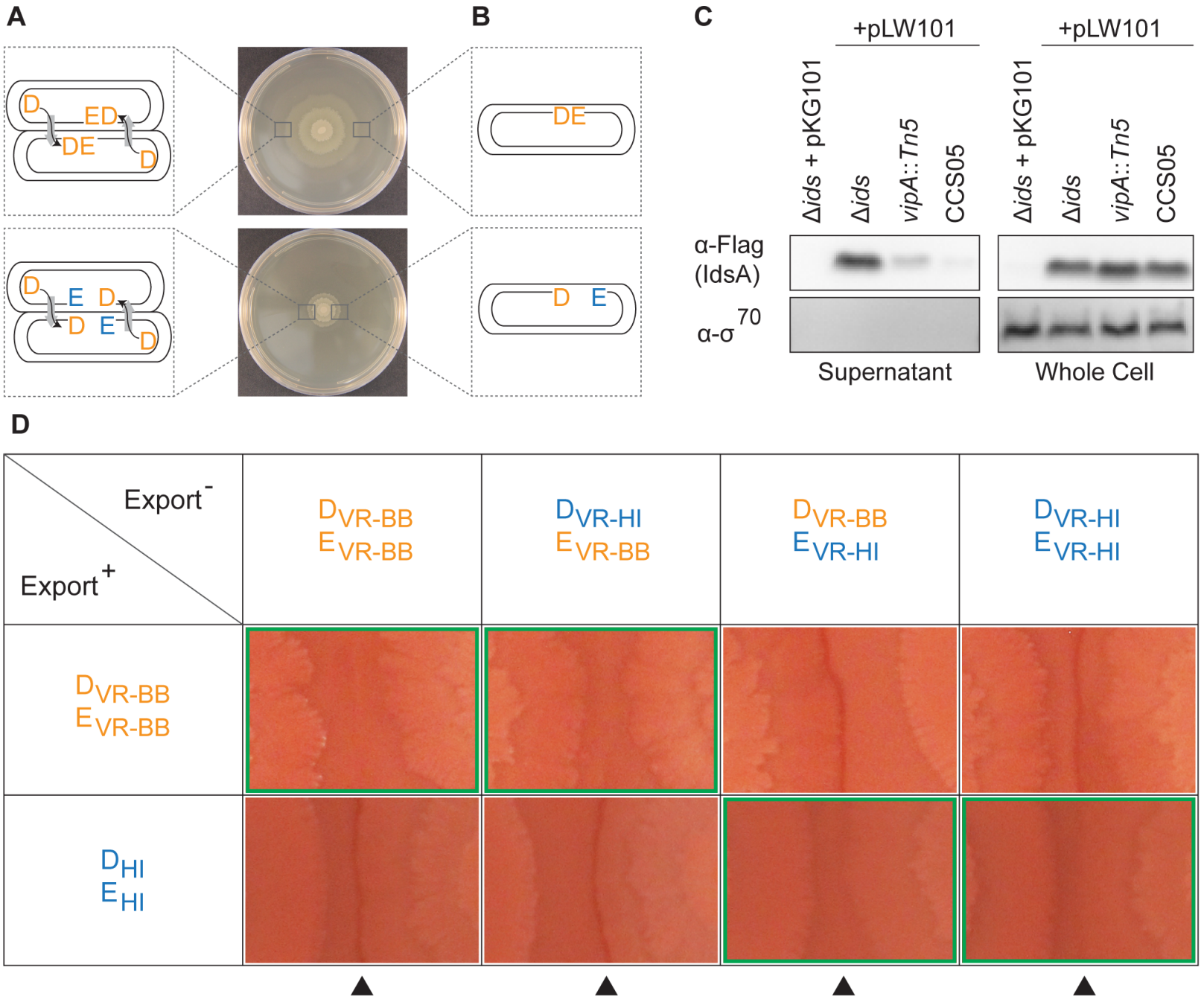
616 **Table 2. LC-MS/MS results of supernatant fractions from approximately 70 to 150 kDa**

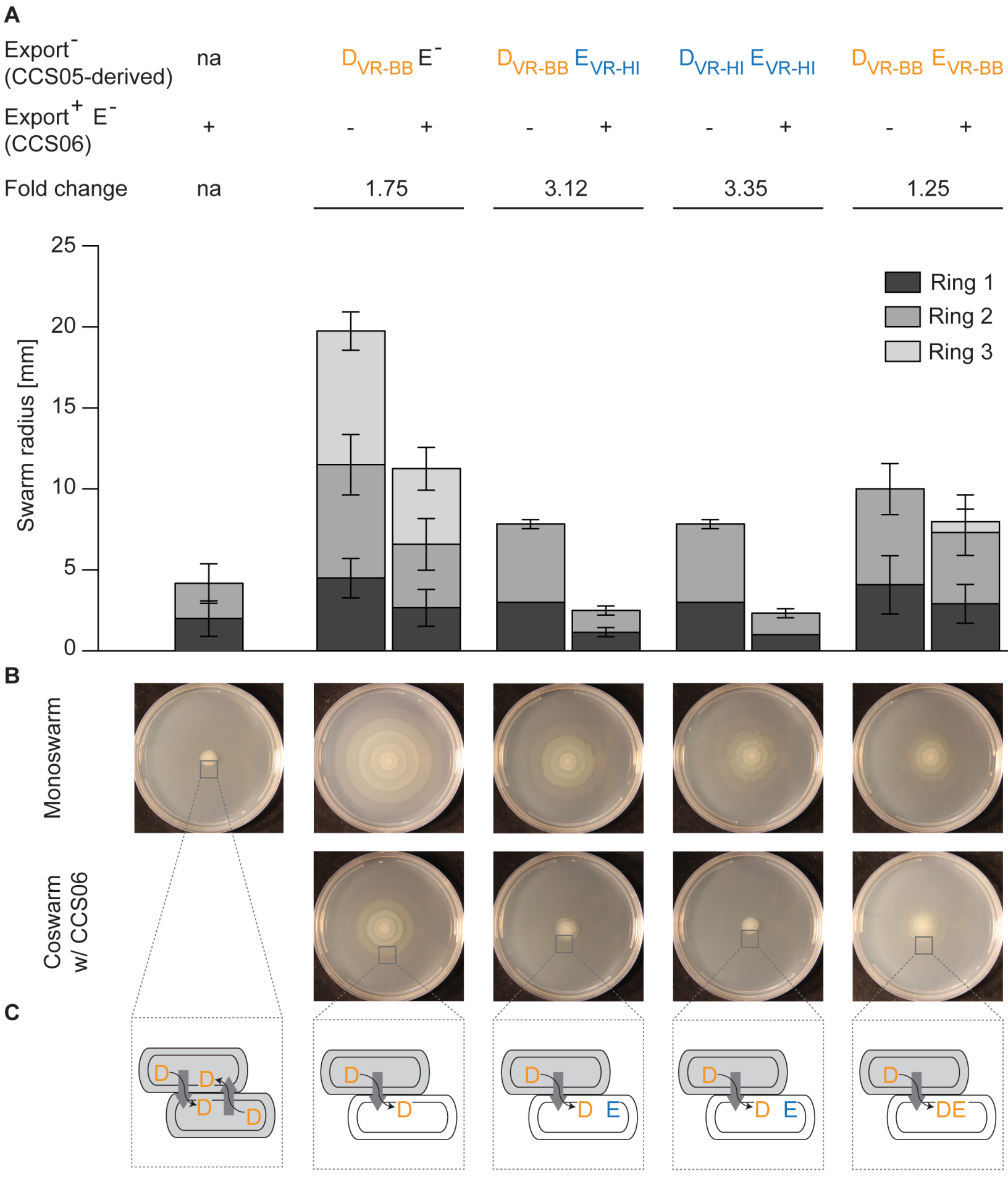
Strain	Protein	Predicted size (kDa)	No. of unique peptides	No. of total peptides	Percent coverage
Δ*ids* + pLMW101 (export-active)	σ^{70}	71.11	3	3	4.05
	IdsB	81.55	5	5	10.65
	IdsD	118.16	2	6	2.32
CCS05 + pLMW101 (export-inactive)	σ^{70}	71.11	2	2	2.91

617


A
Swarm radius [mm]
20
15
10
5
0
Ring 1
Ring 2
Ring 3
CCS#
01
02
03
04
DVR-BB EVR-BB
DVR-HI EVR-BB
DVR-BB EVR-HI
DVR-HI EVR-HI
B
CCS01
CCS02
CCS03
CCS04
C
Viable Cells/Swarm
10^11
10^10
10^09
10^08
10^07
D
Swim diameter [mm]
30
20
10
0
CCS#
01
02
03
04
DVR-BB EVR-BB
DVR-HI EVR-BB
DVR-BB EVR-HI
DVR-HI EVR-HI


A
CCS01
CCS02
CCS03
CCS04
CCS06
Viable Cells/Swarm
10^9
10^8
10^7
10^6
10^5
10^4
10^3
0
2
4
6
8
10
Time [h]
B
Generation time [min]
150
100
50
0
CCS#
01
02
03
04
06
DVR-BB EVR-BB
DVR-HI EVR-BB
DVR-BB EVR-HI
DVR-HI EVR-HI
DVR-BB E


A
B
C
D
DE
ED
DE
D
DE
D E D
D E
D
D E
+pLW101
+pLW101
Δids + pKG101
Δids
vipA::Tn5
CCS05
Δids + pKG101
Δids
vipA::Tn5
CCS05
α-Flag
(IdsA)
α-σ70
Supernatant
Whole Cell
Export-
Export+
DVR-BB
EVR-BB
DVR-HI
EVR-BB
DVR-BB
EVR-HI
DVR-HI
EVR-HI
DVR-BB
EVR-BB
DHI
EHI


A
Export- (CCS05-derived)
Export+ E- (CCS06)
Fold change
na
+
na
DVR-BB E-
- +
1.75
DVR-BB EVR-HI
- +
3.12
DVR-HI EVR-HI
- +
3.35
DVR-BB EVR-BB
- +
1.25
Swarm radius [mm]
0
5
10
15
20
25
Ring 1
Ring 2
Ring 3
B
Monoswarm
Coswarm w/ CCS06
C
D
E
DE